Reference: *Biol. Bull.* **180:** 355–371. (June, 1991)

Embryonic Development of the American Lobster (*Homarus americanus*): Quantitative Staging and Characterization of an Embryonic Molt Cycle

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Abstract. The growth of a single brood of lobsters (*Homarus americanus* Milne-Edwards 1837) maintained at constant temperature is studied from the naupliar stage to hatching, and the sequence of appearance of morphological, anatomical, and behavioral characteristics observed. A percent-staging system based upon Perkins' eye index (1972) is presented, and ten equally spaced embryonic stages are illustrated and characterized at different levels of resolution: whole eggs, dissected embryos, antennulae and telsons. The tegumentary and setal changes in the telson show that a complete molt cycle takes place in the egg starting at about 12% embryonic development (E12%) with the molt of the nauplius into the metanauplius and ending just after hatching when the metanauplius molts into a first stage larva (L1, first zoea). At E30%, the cuticle begins to separate from the setae in the telson; this signals the start of Drach's (1939) stage D_0 of the metanaupliar embryonic molt cycle. At that time, the first sign of organogenesis of the L1, the formation of the endopod of the antennulae, becomes visible; presumed sensory neurons and their axons are observed at the tip of the exopod of the antennulae where a giant sensillum is differentiating. During D_0 the setae of the first larval stage are forming proximally and medially in the bilobed telson under the metanaupliar cuticle. At E90%, these setae are retracting, and the embryo has entered stage D_1. After hatching (E100%), the telson of the free metanauplius (prelarva) shows the characteristics of stage D_{2-3} and ecdysis soon follows. The arrested development observed at constant temperature in the experimental brood occurred at stage D_0 of the metanaupliar molt cycle, whereas development was resumed as the embryos entered stage D_1. These changes in developmental pace from D_0 to D_1 in the embryonic molt cycle are parallel to those occurring in older lobsters (Aiken, 1973). The quantitative staging of lobster development from extrusion to hatching, and the description of the embryonic molt cycle will facilitate future investigations on particular aspects of the embryogenesis of *Homarus* such as neural differentiation.

Received 3 December 1990; accepted 8 March 1991.

Introduction

Studies on lobsters and other crustaceans have made a significant contribution to our understanding of neural organization and the control of behavior (see Wiese *et al.*, 1990). There is increasing interest in examining the ontogenesis of particular behaviors and the cellular architecture that is the basis for those behaviors (Kravitz, 1988; Govind, 1989; Sandeman and Sandeman, 1990). Research on neural development at the embryonic level in *Homarus* is flourishing (Cole and Lang, 1980; Beltz and Kravitz, 1987; Beltz *et al.*, 1990; Helluy and Beltz, 1990; Meier and Reichert, 1990), but progress has been limited by the lack of adequate documentation on the general development of this organism in the egg, as well as by the absence of a staging system for the total embryonic period. These two problems are addressed in this paper.

Recent developmental studies in *Homarus* have dealt primarily with the perihatching period (Davis, 1964; Ennis, 1975; Charmantier and Aiken, 1987), and larval and postlarval life (Phillips and Sastry, 1980; Charmantier, 1987), whereas most of the literature concerned with the prehatching period dates back to the nineteenth century (Bumpus, 1891; Herrick, 1895). The latter studies are a remarkable achievement of patient and detailed observation and are illustrated by elegant drawings (Herrick, 1895), but the modern microscopic and photographic methods used in this study are necessary to provide added resolution. The nineteenth century studies also tend to focus on early embryogenesis while providing little or no information about middle and late development in the

egg, and the embryonic molt cycle. A deeper knowledge of lobster embryology could also provide more insight and understanding of studies that examine particular aspects of development, such as the influence of temperature on growth rate (Templeman, 1940; Perkins, 1972), population dynamics (Schuur *et al.* 1976; Hepper and Gough, 1978), the chemical composition and calorific content of the eggs (Pandian 1970a, b; Sasaki, 1984; Sasaki *et al.* 1986), or the differentiation of particular organs or systems, such as heart and gut (Burrage, 1978; Burrage and Sherman, 1979), and, again, nervous system.

The principal features involved in the reproduction and early development of the lobster *Homarus americanus* are well known. After copulation, spermatozoa are stored by the female for several months until oviposition and fertilization occur (Aiken and Waddy, 1980). In New England waters, egg development spans about 10 months, from egg extrusion in July or August to hatching the following May or June (Bumpus, 1891; Herrick, 1895). Following extrusion, the eggs are carried on the abdomen of the mother, attached to the pleopods. *Homarus* has relatively large, telolecithal eggs. Superficial cleavage leads to the formation of a blastoderm, and the central mass of yolk remains undivided (Bumpus, 1891). After only a few days, the naupliar organization is apparent. The nauplius, which is a developmental hallmark of crustaceans, is characterized by the presence of a median eye and three pairs of appendages: the antennulae, antennae, and mandibles (Shiino, 1988). The metanauplius arises from the differentiation and growth of the postmandibular appendages. *Homarus* hatches as a mature metanauplius (prelarva, prezoea) that molts rapidly into the first larval stage (Davis, 1964). There are three pelagic larval stages swimming with the feathery exopodites of six pairs of thoracic limbs. A metamorphosis leads to the formation of a postlarva (fourth stage) with most of the adult characteristics (Charmantier, 1987). The postlarva, which swims in a fully extended posture using its pleopods, later settles on the substrate. The duration of larval life, in the order of a few weeks, depends largely on temperature.

For the present study, behavioral, morphological, anatomical, and morphometric data were gathered from whole eggs and dissected embryos. A percent-staging scheme using the size of the pigmented area in the lateral eyes [the eye index (Perkins, 1972)] was adopted. Subsequently, ten equally spaced developmental stages were documented in detail with the eggs of different females. Particular attention was given to the growth and differentiation of the antennulae and telson. The antennulae, which are lined with aesthetascs (olfactory sensilla) in postembryonic animals from second larval stage on, were examined to gain insight into the ontogeny of the olfactory sensory apparatus. The telson was studied to elucidate how the round bilobed telson of the embryo is transformed into the triangular telson of the first larval stage.

Materials and Methods

Lobster and egg maintenance

Egg-bearing female lobsters *Homarus americanus* (Crustacea, Malacostraca, Decapoda, Reptantia, Astacidea, Nephropidae) were obtained from the Massachusetts State Lobster Hatchery on Martha's Vineyard, Massachusetts, and kept in recirculating artificial seawater. In addition, eggs detached from the mother's abdomen were provided by the New England Aquarium in Boston, Massachusetts, where lobsters were reared in filtered, temperature-controlled seawater. These detached eggs were maintained in our laboratory in free-floating net enclosures in artificial seawater. We found that hanging the clumps of eggs with surgical thread, and allowing them to float, led to good survival rates. Three tanks were maintained at temperatures of 10 ± 2°C, 18 ± 2°C, and 20 ± 2°C, to slow or accelerate the rate of development of the eggs, at a salinity between 27 and 32 ppt in a 12:12 light:dark cycle.

The experimental brood

We have not had any success promoting egg extrusion in females held in recirculating tanks, probably because of the variety of complex environmental factors necessary for this event (Waddy and Aiken, 1984). Therefore, in mid-October, the egg-bearing female containing the youngest eggs was chosen from a collection of approximately 200 gravid females collected by fishermen for the State Lobster Hatchery. The earliest stage observed in the experimental brood was a cleavage stage. The approximate date of extrusion was calculated as follows: in Templeman's (1940) experiments, the period from the late nauplius to the first appearance of pigment in the lateral eyes (26 days) lasted about 45% of the time required for the development from extrusion to appearance of eye pigment (58 days) at 12–13°C, and 41% (11/27) in Herrick's experiments (1895, p. 56) at 21°C. In the lobster (Templeman, 1940; Perkins, 1972) and in insects (Bentley *et al.*, 1979), developmental events are more condensed or expanded in time depending on temperature, but the proportion of the total duration of embryogenesis devoted to each developmental event does not change with temperature. In the present study at 18°C, the development from late nauplius to the first appearance of eye pigment took 9 days; therefore, by extrapolation from the data of Templeman (1940) and Herrick (1895), the period from extrusion to first appearance of eye pigment would be predicted to last 20–22 days. Thus, the estimated date of extrusion was calculated to be 21 days prior to the appearance of eye pigment. Note that extrusion did occur in the wild in water at seasonal temperatures.

Observations were made on the experimental brood kept at 18 ± 2°C for five months (mid-October to mid-

March, see Table I). The female died in mid-January, when the eggs were at 66% development; she was stripped of eggs and the spawn was suspended in nets in the tank. The eggs were agitated daily to try to replace the vigorous beating of the pleopods of the mother. In the experimental brood, the majority of the eggs attained the hatching stage but very few actually hatched into free metanaupliae; still fewer molted into first larval stages. Those larvae that did emerge were perfectly normal animals. The smoothness of growth curves of the experimental brood (see Results) and numerous observations on the progeny of other females confirmed that the free eggs of the experimental brood followed a normal course of development after the death of the mother.

Five live eggs from the experimental brood were examined every two or three days for the first two months, then once a week until hatching. As soon as the heart was formed, the heart beat was confirmed in each embryo to ensure that the observed eggs were alive. During each observation period, the width and length of the pigmented area in the lateral eyes (Fig. 1) and the greatest axis of the egg were measured in intact eggs; following dissection, the length of the cephalothorax was measured ventrally from the median eye to the anterior margin of the abdomen (Fig. 2). Behavioral observations, such as antennal twitching or tail flipping during dissection, were also noted. Photographs of whole eggs and dissected embryos were taken with a Zeiss stereomicroscope.

Developmental staging system

A developmental scale was designed that used the eye index (Perkins, 1972) as a marker of developmental progress. The eye index is defined as the average of the length and the width of the brown screening pigment spot (in micrometers) in the lateral eyes. The first measurable eye pigment spot had an eye index of 70 μm (Perkins, 1972; present study). Therefore, development prior to the appearance of eye pigment was characterized using time rather than the eye index. The estimated duration of development of the experimental brood was 159 days (Table I). Eye pigment first appears at 13.2% of the total time from extrusion to hatching, while the eye index at first appearance of pigment (70 μm) is 12.2% of the eye index of the experimental brood at hatching (578 μm) (Table I). These values indicate that there is little difference during early embryogenesis between staging based on time and that based on the eye index; time-staging was used prior to, and eye index-staging after 15% development (Table I). Later in embryogenesis, because of the period of developmental arrest (see Results), staging based upon time is no longer valid; the morphometric marker (eye index) must then be used.

Table I

Dates of observation of the experimental brood of Homarus americanus *maintained at 18°C, age, eye index, and percent-staging system. The dotted line signals the transition between percent of total time from extrusion to hatching and percent of eye index at hatching*

Date 88–89	Embryonic age (days)	Eye index (μm)	Stage (%)
10–08	0		0
10–16	8		5.3
10–18	10		6.3
10–20	12		7.6
10–23	15		9.4
10–25	17		10.7
10–27	19		12.0
10–29	21	70.6	13.2
10–31	23	72.5	14.5
11–02	25	98.0	17.0
11–04	27	139.2	24.1
11–06	29	145.0	25.1
11–08	31	156.8	27.1
11–11	34	160.7	27.8
11–18	41	213.6	37.0
11–24	47	248.9	43.1
12–01	54	282.2	48.8
12–08	61	307.7	53.2
12–15	68	329.3	57.0
12–23	76	352.8	61.0
12–29	82	366.5	63.4
01–05	89	386.1	66.8
01–12	96	382.2	66.1
01–19	103	425.3	73.6
01–26	110	447.0	77.3
02–02	117	441.0	76.3
02–09	124	458.6	79.3
02–16	131	466.5	80.7
02–23	138	474.3	82.1
03–02	145	474.3	82.1
03–09	152	542.9	93.9
03–16	159	578.2	100.0

Characterization of ten embryonic stages and of the embryonic molt cycle

Following the adoption of the percent-staging system, eggs from different broods were studied in more detail at every 10% increment in development. The eye index at hatching was estimated at 570 ± 20 μm (see Discussion), and therefore each 10% increment in developmental maturity was characterized by an increase of 57 μm in the eye index. The stage described as 10% was reached on day 16 in the experimental brood: the late egg-nauplius. Eggs were also examined when their eye indices measured 114 ± 6 μm (E20%), 171 ± 4 μm (E30%), 228 ± 7 μm (E40%), 285 μm ± 14 (E50%), 342 ± 11 μm (E60%), 399 ± 17 μm (E70%), 456 ± 5 μm (E80%), 513 ± 20 μm (E90%), 570 ± 20 μm (E100%). The varying range for each stage reflected embryo availability and limitations

imposed by the precision of the ocular micrometer. Twenty micrometers represents 3.5% of the total development scale. To characterize each of the ten stages and the early postembryonic stages, the same protocol described earlier for the experimental brood was used. Photographs of whole eggs (Figs. 3, 4) and dissected embryos (Fig. 5) were taken with a Zeiss stereomicroscope. In addition, antennulae (Fig. 6) and telsons (Fig. 7) were also severed, examined fresh, and photographed using a Zeiss IM35 photoinvertoscope equipped with modulation contrast optics (Hoffman, 1977).

Drach (1939) and Drach and Tchernigovtzeff (1967) designated the phases of ecdysis in crustaceans by letters from A to E: A and B are the postmolt periods, C the intermolt, D the premolt period, and E the ecdysis proper. In the present study, this system was used to characterize the molt cycle of *Homarus* that occurs within the egg envelopes. The period of the embryonic molt cycle was determined by matching setal changes in the telson of embryos (Figs. 7 and 8) with the setal changes in the telson previously documented in larvae of *Homarus americanus* (Rao *et al.*, 1973; Sasaki, 1984) and in the pleopods in juveniles (Aiken, 1973; 1980) during molt cycles. The subdivisions of stage D (D_0, D_1, D_{2-3}) and their distinguishing features are those described by Sasaki (1984).

Terminology

More than 70 terms have been used to refer to the various embryonic and larval stages of decapods (Gore, 1985). The form that arises from the differentiation and growth of the postmandibular appendages in the American lobster egg has been called a "post-nauplius" by Herrick (1895) or "postnauplius" by Helluy and Beltz (1990). In the present study "metanauplius" is used, a term commonly assigned to the form developing just past the naupliar stage (Wear, 1974; Williamson, 1982; Shiino, 1988). The form that is released from the egg envelopes and rapidly molts into the first larval stage is usually called a "prelarva" or "prezoea"; however, the term "mature metanauplius" seems more biologically relevant (see Discussion). The first larval stage of *Homarus* is sometimes referred to as a "mysis" (Shiino, 1988) based on the number of its appendages, or a first "zoea" because it locomotes with its thoracic appendages (Anderson, 1982; Williamson, 1982). Finally, "embryogenesis," "prehatch," and "egg development" are used interchangeably, although the latter part of egg development in *Homarus* is devoted to larval organogenesis rather than to embryogenesis strictly defined.

Results

I. Timing of development, sequence of events, and characterization of embryonic and early postembryonic stages

In the following account of the embryogenesis of *Homarus americanus*, the sequence of developmental events and morphometric data on eye index (EI) and cephalothoracic length (Figs. 1, 2) were obtained by studying the experimental brood, whereas the illustration and characterization of equally spaced embryonic and early postembryonic stages was achieved by studying many clutches

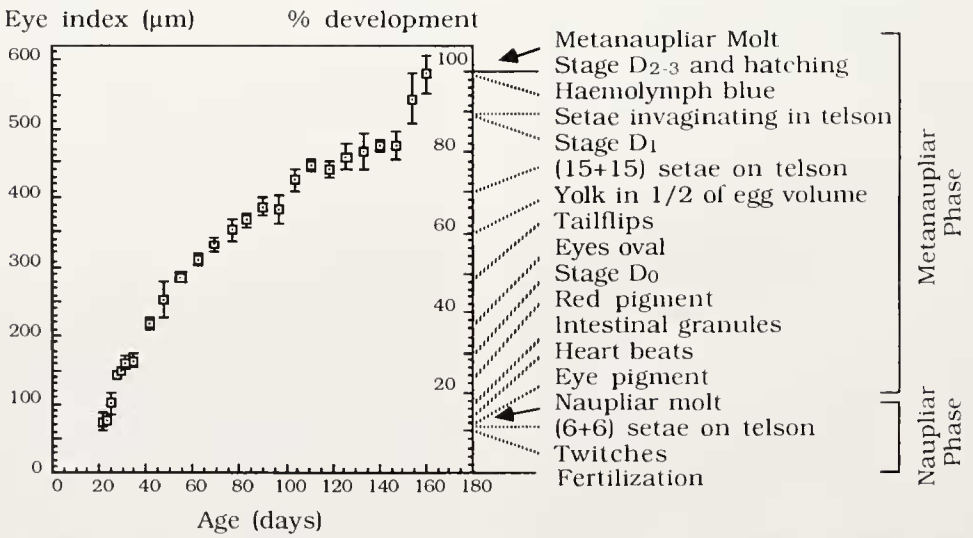


Figure 1. Eye index *versus* age of the experimental brood of *Homarus americanus*, maintained at 18°C. Developmental landmarks are indicated along a percent-scale based on the eye index. Perkins' eye index (1972) is the mean of the length and the width of the screening pigment spot in the lateral eyes. Each data point represents the mean of measurements on five individuals of the experimental brood ± one standard deviation.

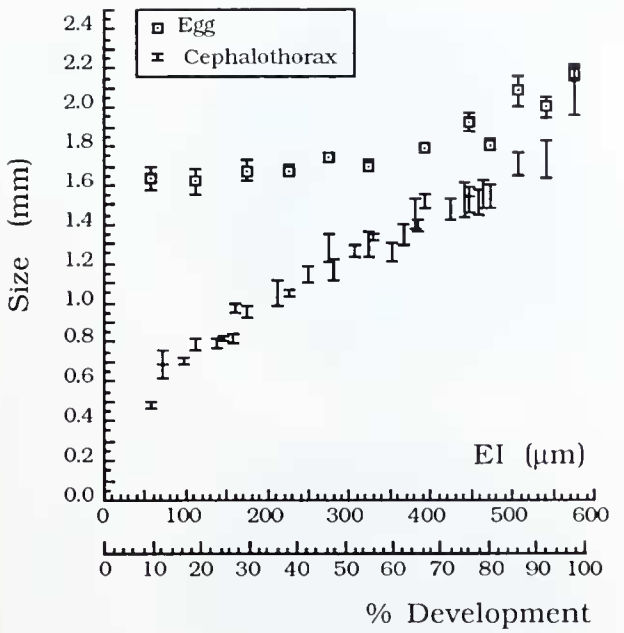


Figure 2. Greatest axis of egg and cephalothoracic length *versus* eye index and percent-development scale, in *Homarus americanus*. Greatest axis of egg: each data point represents the mean of five measurements ± one standard deviation (data points from different broods). Cephalothoracic length: the length of each bar represents one standard deviation on each side of the mean of five measurements (all individuals from experimental brood).

of eggs at different levels of resolution [whole eggs (Figs. 3, 4), dissected embryos (Fig. 5), antennulae (Fig. 6), and telsons (Figs. 7, 8)]. The stage of appearance of developmental events is expressed in percent-development of total embryogenesis. The percent-staging scheme is explained in "Materials and Methods." Dates of observation of the experimental brood, age, eye index, and percent-staging system are related in Table I and in Figure 1. The metanaupliar molt cycle is described in the second part of "Results."

Sequence of events prior to 10% development. The first organized structure to appear at the surface of the green yolk at 5% development (E5%, estimated day 8 of the experimental brood) is a typical crustacean nauplius with three pairs of appendages: the antennulae, the antennae, and the mandibles. The mandibles are first visible as two dots medial to the endopods of the antennae. This is equivalent to stage "M" of Bumpus with the eye lobes and the thoracoabdominal process still undefined. At E6% (day 10), the optic lobes appear as a white cloud of cells, and the thoracoabdominal process is clearly outlined. At E8% (day 12, stage "N" of Bumpus) the optic lobes are also delineated, and the embryo is easily separated from the yolk. The dorsal side of the embryo is apposed to the yolk, and the abdomen grows folded on the ventral side of the thorax. The tip of the abdomen reaches the level of the mandibles and is beginning to part medially at E9% (day 15, stage "O" of Bumpus); the buds of four pairs of post mandibular appendages line the trunk.

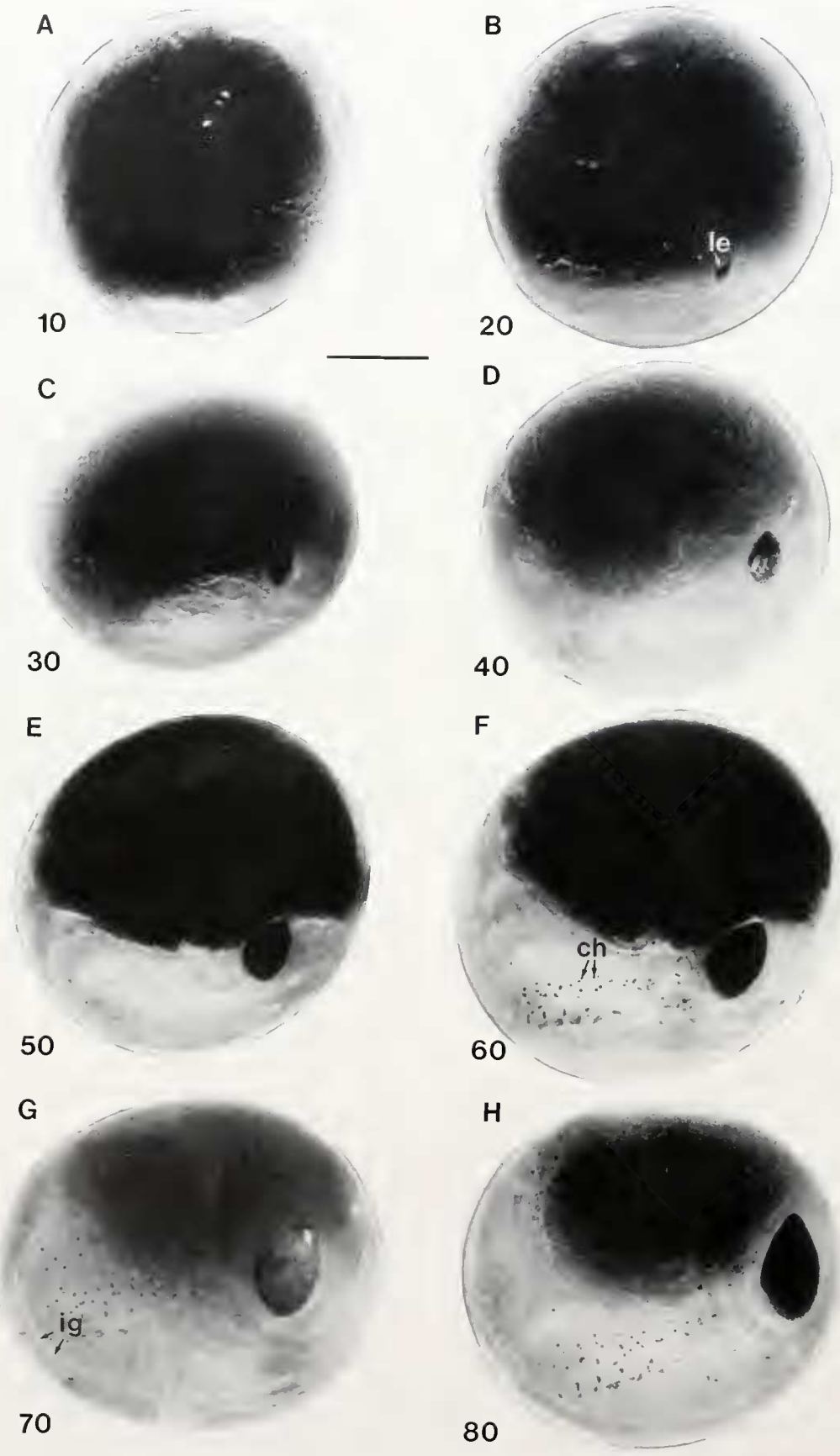
10% development (no eye pigment present: Figs. 3A, 5A, 6A, 7A). At E10%, the yolk occupies approximately 95% of the volume of the egg (Fig. 3A) whose greatest axis measures about 1.6 mm. The antennulae are uniramous and end with five setae whereas the antennae are biramous with five setae at the tip of the exopods and three setae at the tip of the endopods (Fig. 6A). The extremity of the abdomen nearly reaches the level of the labrum; the telson is beginning to part but setae are not yet present at its tip. This stage is equivalent to stage "O" of Bumpus (1891).

Sequence of events from 10% to 20% development. At E11% (day 17) at least 8 appendages are formed past the mandibles and at about this time, the first twitches in the two pairs of antennae occur upon dissection. Pigment is then visible in the median eye under the compound microscope. At about E12%, when pigment is already visible in the median eye but not yet in the lateral eyes, an embryonic molt occurs (see II, below). At least two envelopes surround the antennulae (Fig. 6B), and one envelope is stretched at the tip of the six setae (6 + 6) on each side of the telson indicating the occurrence of a molt (Figs. 7B, 8A). At about E13% (day 21), pigment is seen in the lateral eyes as a small dark crescent lining the posterior part of the lobes; the eye index at that stage is approximately 70 μm. At E14% (day 23), the first heartbeats are seen in approximately 10% of the embryos examined. By E17% (EI 98) heartbeats are seen in half of the eggs, and small refringent granules are present in the intestine.

20% development (EI = 114 μm; Figs. 3B, 5B, 6C, 7C). Muscular twitches are readily observed in the antennae, around the mouth, and in the abdomen, and the heart is beating. Between 10 and 20% of embryonic development, the nauplius has molted into a metanauplius (see II, below). This stage is equivalent to stage "P" of Bumpus (1891), defined as the stage when the tips of the third pair of maxillipeds reach the point of insertion of the antennulae and the telson reaches the level between the mouth and the median eye. The telson is provided with 6–7 setae on each of the two lobes (Fig. 7C).

Sequence of events from 20% to 30% development. At E24% (EI 139), red pigment spots (chromatophores) appear on each side of the brain. At E25% (EI 145) the telson reaches the anterior edge of the optic lobe (stage "Q" of Bumpus). The full complement of appendages present in the metanauplius is formed by E27% (EI 157), and by E28% (EI 161) the telson reaches beyond the optic lobes.

30% development (EI = 171 μm; Figs. 3C, 5C, 6D, 7D). The cephalothorax of the embryo is nearly 1 mm long. Red chromatophores are present on each side of the brain. A cluster of presumed sensory neurons has formed in the exopod of each antennula and their axons follow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the anterior edge of these appendages in a bundle of a few micrometers (Fig. 6D). Serial plastic sections have shown that the bundle of axons projects to the olfactory lobes (in the deutocerebrum), which measure about 40 μm in diameter at that stage (unpub. results). The cluster of neurons is very similar to the cluster of bipolar sensory neurons that innervate each aesthetasc (olfactory sensillum) in the antennulae of spiny lobsters (Grünert and Ache, 1988). At E30% also, the endopod of each antennula tipped with a pointed seta, is visible under the cuticle (Fig. 6D). The endopods are freed after hatching when the metanauplius molts into a first larval stage (L1). The appearance of the endopod of the antennulae is the first visible sign of the formation of the L1 under the cuticle of the metanauplius. All postmandibular appendages are present and the trunk is lined with six pairs of prominent appendages: a pair of third maxillipeds and five pairs of walking legs. During the metanaupliar phase, the trunk appendages, which are uniramous, cannot be separated from each other. The tips of the third maxillipeds reach a level between the point of insertion of the antennulae and the anterior edge of the optic lobes whereas the telson is at the level of the anterior edge of the optic lobes [stage "Q" of Bumpus (1891)]. In the telson, the metanaupliar cuticle begins to separate from the side of the setae but the tip of these setae is still attached to the cuticle (Fig. 7D); this signals the start of the premolt stage D_0 of the metanaupliar molt cycle (see II, below).

Sequence of events from 30% to 40% development. Stage "R" of Bumpus is reached between E30% and E37% when the tip of the third maxillipeds is at the level of the antennae and the telson grows beyond the optic lobes. By E37%, the eye pigment spots have become oval rather than crescent-shaped, and red pigment granules line the sides of the nerve cord.

40% development (EI = 228 μm; Figs. 3D, 5D, 6E, 7E). A giant sensillum (260 μm) is visible as a long straight rod inverted at the tip of the exopod of each antennula. Setae are present at the extremities of trunk appendages. Red chromatophores are seen on the sides of the nerve cord, on the anterior edge of the optic lobes, and on the growing carapace. The third maxillipeds reach the anterior edge of the optic lobes, and the telson reaches anteriorly to the optic lobes. This stage is more advanced than stage "R," the most advanced stage described by Bumpus (1891).

Sequence of events from 40% to 50% development. The red chromatophores have invaded the appendages and the growing carapace by E43% and the abdomen by E49%.

50% development (EI = 285 μm; Figs. 3E, 5E, 7F). By E50%, some embryos perform very clear tailflips after removal of egg envelopes; also, the first caeca of the paired digestive glands (hepatopancreas) are seen, with the stereomicroscope, at the anterior end of the midgut where it comes in contact with the mass of yolk. The rostrum of the differentiating L1 is folded ventrally between the optic lobes and is visible upon dissection. The gap between the cuticle and the six or seven most distal and lateral setae on each side of the telson has widened, but the tips of these setae are still in contact with the cuticle (Fig. 7F). Other setae are growing more medially and more proximally beneath the cuticle of the telson. By now, the distal ends of the third maxillipeds, as well as the telson, reach anteriorly to the level of the optic lobes.

Sequence of events from 50% until hatching. There are no obvious changes in the general external morphology of the embryo from E50% until hatching (E100%). However, the embryo grows dramatically and the structures typical of the L1 are forming progressively beneath the cuticle of the metanauplius.

60% development (EI = 342 μm; Figs. 3F, 5F, 6F, 7G). At this stage the yolk occupies about half the volume of the egg. At least 10 setae are formed on each side of the telson (Fig. 7G).

70% development (EI = 399 μm; Figs. 3G, 5G, 7H, 8B). The full complement of setae (14 or 15 on each side) of the first larval stage is present on the telson under the metanaupliar cuticle; the median spine begins to differentiate (Figs. 7H, 8B).

80% development (EI = 456 μm; Figs. 3H, 5H, 6G, 7I). In the telson, only the most medial setae are in contact with the metanaupliar cuticle. These setae have not yet assumed the shape of spines, and the embryos are still in stage D_0 of the metanaupliar molt cycle (Fig. 7I).

90% development (EI = 513 μm; Figs. 4A, 5I, 6H, 7J, 8C). The egg is now enlarging rapidly, and its largest axis measures about 2.0 mm (Fig. 2). The yolk is turning yellow (Fig. 4A). The telson manifests a number of dramatic changes (Figs. 7J, 8C). The cuticle has lifted entirely from the setae and also from the epidermis on the lateral sides of the telson. The two most lateral setae are now pointed and sharp like spines, and they begin to retract. About a third of each seta is visible beneath the tegument. The

Figure 3. Unfixed, intact eggs of *Homarus americanus* at (A) 10, (B) 20, (C) 30, (D) 40, (E) 50, (F) 60, (G) 70, and (H) 80% embryonic development. The figures in the lower left corners refer to the percentage of development. In all photographs, the dorsal side is at the top, and the head and telson of the embryo are on the right. At 10% development (E10%), the embryo is seen as a small halo at the bottom part of the egg. The eye pigment is visible in the lateral eyes (le) by E20%. The red chromatophores (ch) already present by E40% are labeled at E60%. The intestinal granules (ig) are particularly clear at E70%. Scale bar: 500 μm.

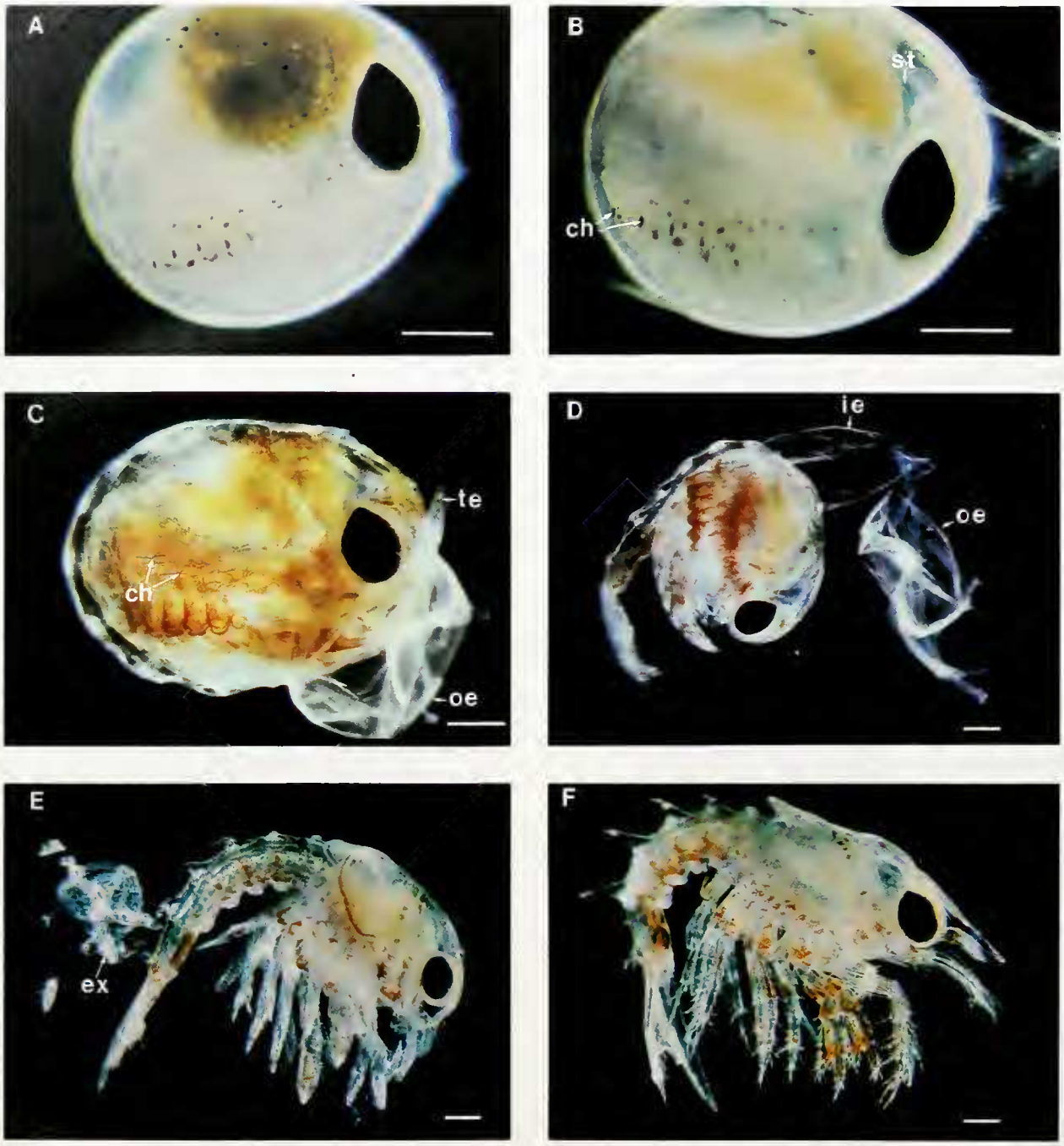


Figure 4. Perihatching development of *Homarus americanus*. In all these photographs of unfixed specimens, dorsal side is at the top, and anterior is right. (A) 90% embryonic development. (B) Embryo just prior to hatching (100%, blue embryo): note the blue tinge of the hemolymph, the blue stomach (st) and the red chromatophores (ch) in which the pigment is still concentrated. (C) Hatchling: the outer (oe) egg envelope has burst, and the telson (te) is piercing the inner egg envelope; the red pigment has spread in the star-shaped chromatophores (ch). (D) The metanauplius (prelarva, prezoea) is now free of both outer (oe) and inner (ie) egg envelopes. (E) Early first larval stage (first zoea): the exuvia (ex) of the metanauplius has been sloughed. (F) Mature first larval stage: rostrum, abdominal spines, and other acuminate structures are now erect. Scale bars: 500 μm.

epidermis forms papillae around each seta, and appears scalloped. Retraction of setae and scalloped epidermis are characteristic of stage D_1 (Sasaki, 1984).

100% development (EI = 570 μm; Figs. 4B, 7K). At this stage, just prior to hatching, the egg (2.2 mm) is brightly colored (Fig. 4B). The stomach is deep blue and the hemolymph pale blue. The yolk, which has been nearly entirely absorbed, is yellow or pale green. The two pairs of yolk caeca that were filling the egg earlier are attached to the digestive tube dorsally by this time, between the pyloric stomach and the numerous tubular digestive glands. The bilateral spines of the telson are entirely retracted, whereas the setae are only partially so.

Eclosion of the metanauplius (hatchling) (Figs. 4C, 6I, 7L, 8D). The outer egg envelope has burst. The red pigment disperses in star-shaped chromatophores (Fig. 4C). The giant sensillum is everted and projects from the exopods of the antennulae, but is still confined within the cuticle of the metanauplius (Fig. 6I). The spines and setae of the telson begin to expand (Figs. 7L and 8D). The epidermis becomes very distinct and forms a pronounced bulging around the invaginated setae; these are two characteristics of stage D_{2-3} of Sasaki (1984).

Free metanauplius (prelarva, prezoea; Fig. 4D). The metanauplius is freed of the two external egg envelopes; it is mostly still, but occasionally performs strong tail-flips. These movements presumably facilitate the molting process. Within hours after the egg membranes are shed, the metanauplius molts into a first larval stage.

Molt of the metanauplius and emergence of first larval stage (Figs. 4E and F, 6J, 7M). The exuvia peels away from the metanauplius. Slowly the rostrum, the abdominal spines, and all the other acuminate structures become erect. The telson opens like a fan into a triangular structure (Fig. 7M). The setae evaginate. The endopod of each antennula is released (Fig. 6J), as well as the feathery exopodites of the six pairs of thoracic appendages. Each antennula at hatching has one giant sensillum (550 μm) and three setae at the tip of the exopod, and one seta at the tip of the endopod (Fig. 6J) as reported by Herrick (1895). In brief, the curvaceous metanauplius molts into an angular larva ready to assume a pelagic life.

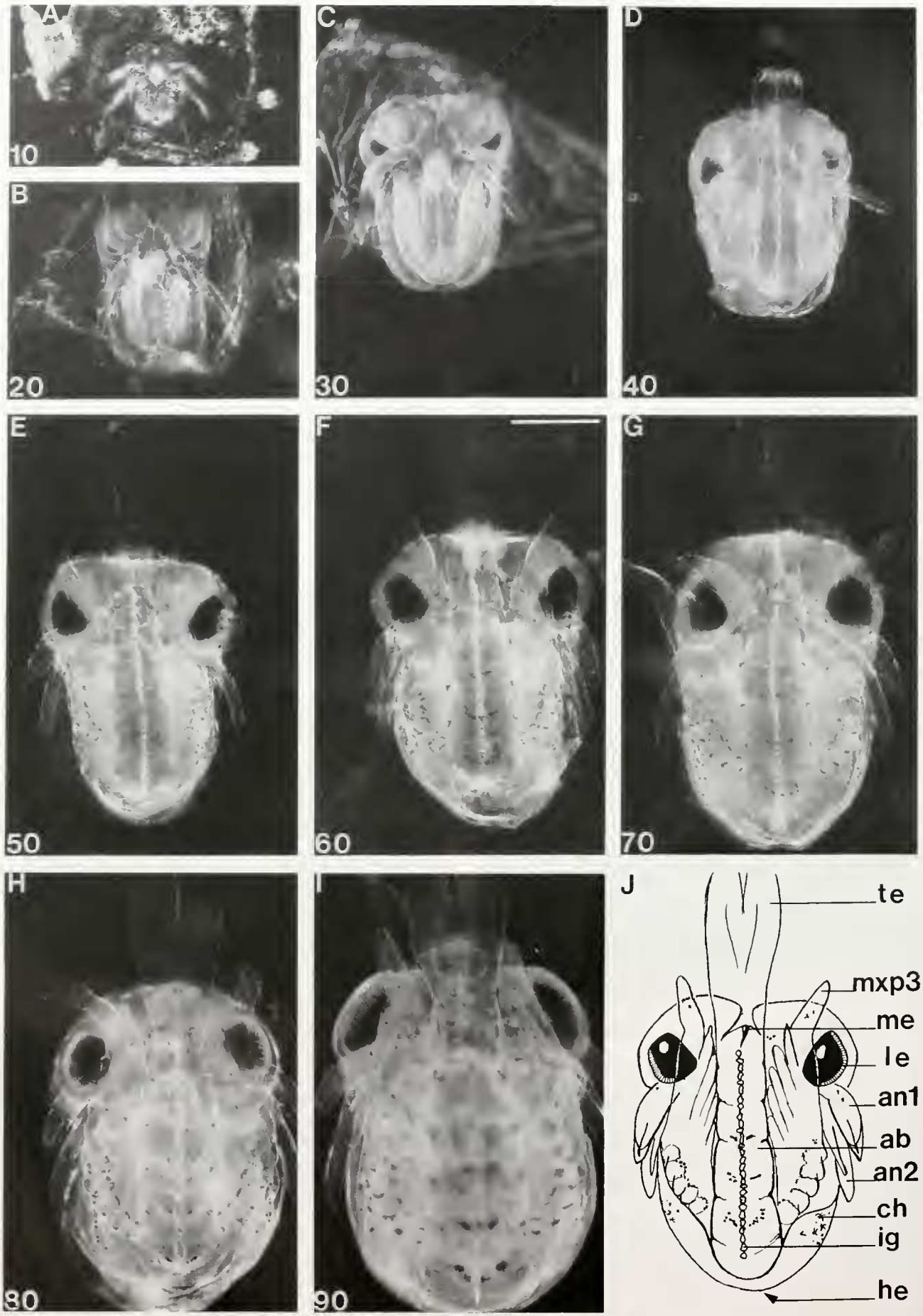
II. Metanaupliar embryonic molt cycle, growth curves and developmental plateau

Metanaupliar embryonic molt cycle. At about E12%, an envelope is seen enshrouding the telson (Figs. 7B and 8A), stretched at the tips of the 12 (6 + 6) bilaterally paired setae on the telson of the nauplius. This envelope is thought to be the exuvia of the naupliar stage; it is flat and was formed in the nauplius when the tip of the abdomen had not yet acquired any setae. The metanauplius that emerges at the naupliar molt has been forming during the naupliar stage. From this molt until the emergence of the first larval stage after hatching (metanaupliar molt), a complete molt cycle is observed in the setal changes of the telson. The cuticle begins to lift away from the telson at about E30% (Fig. 7D) when the metanauplius enters stage D_0. The shape of the 6 + 6 setae on the telson of the metanauplius is well defined on this cuticle. Setae form then medially and proximally, and by E70% (Fig. 7H, 8B), the full complement of setae of the first larval stage (15 + 15) is formed. Between E80% (Fig. 7I) and E90% (Fig. 7J), dramatic setal and tegumentary changes occur as the metanauplius enters stage D_1. Particularly striking is the transformation of the most lateral setae into straight and sharp spines (compare Figs. 7I and J, 8B and C). These spines are invaginating as well as all the setae. In addition, the epidermis becomes scalloped, and the cuticle lifts from the sides of the telson. Just prior to hatching (E100%), retraction of spines and setae is maximal. When the outer egg envelope ruptures, the bulging of the epidermis around the setae is pronounced, and the metanauplius has entered stage D_{2-3}. After hatching, the metanauplius molts into the first stage larva and the cuticle which is discarded still has the typical metanaupliar shape with the imprint of the (6 + 6) metanaupliar setae.

Growth curves and developmental plateau. The greatest axis of the egg increases gradually from 1.6 mm at E10% to about 1.8 mm at E80% and more rapidly to 2.2 mm at hatching (Fig. 2). In the experimental brood raised at 18°C, both the eye index (Fig. 1) and the cephalothoracic length showed a logarithmic growth from the first time these variables could be measured to approximately day 110. In Figure 2, cephalothoracic length appears linear because it is expressed as a function of the eye index. The eggs of the experimental brood reached a developmental plateau at an eye index of about 474 (E82%). Until this stage, development of the eggs was synchronous with little interindividual variability within the brood. This was shown by the low standard deviation of the eye index and of the cephalothoraxic length (Figs. 1, 2). Until 82%, all eggs were "green" and taken at random. However, after E82%, the population was no longer homogeneous, and the naked eye could distinguish by size and color three categories of eggs: "green," "yellowish" (Fig. 4A), and "blue" (Fig. 4B). In addition, the eggs hatched over a period of about a month, again indicating significant variability between eggs in a single brood. After E82% it was no longer possible to choose eggs randomly for observation; to assign an approximate age to each of these stages, "yellowish eggs" were examined on day 152 when the majority of eggs had reached this stage, and "blue eggs" (the hatching stage) were examined a week later. Individual eggs took about two weeks to change from "green" to "blue" eggs at 18°C.

Discussion

In the present paper, different aspects of the embryonic development of *Homarus americanus* are examined from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the formation of the naupliar stage until the emergence of the first larval stage. In the discussion that follows, the percent-staging scheme presented is compared to that used in other invertebrate systems, and anatomical and morphological observations of earlier authors are related to that staging system. The developmental plateau in the growth curve of the eggs is discussed in the context of the embryonic molt cycle. The occurrence of embryonic molt cycles in other crustaceans is reviewed and the significance of the prelarva debated.

Staging system

Embryonic studies on invertebrates have used staging systems based upon particular developmental events, and arbitrary notations such as letters or figures (Bumpus, 1891; Figueiredo and Barraca, 1963; Fernandez, 1980) to demarcate stages. Nevertheless, when intermediate stages are likely to be needed, a continuous rather than incremental staging system, and in particular a percent-staging system, is more flexible and communicable (Bentley *et al.*, 1979). In addition, a percent-staging method takes into account the entire embryonic life of the organism without ignoring periods when no particular biological events seem noticeable. For example, Bumpus (1891), Herrick (1895), and Templeman (1940) observed *Homarus* eggs only until the lateral eye pigment spots became oval, about 40% embryonic development in the present study.

Age in warm-blooded animals is generally a good indicator of the stage of development, but in invertebrates the rate of development is strongly dependent on temperature. To circumvent this problem, some methods have relied on a percent-staging scale of total embryonic time (*Schistocerca:* Bentley *et al.*, 1979; *Helisoma:* McKenney and Goldberg, 1989; *Cherax:* Sandeman and Sandeman, in press). In these studies, the staging scale is "calibrated" at a given constant temperature. The time from fertilization to hatching is then transformed into a percent-staging scale. This scale is applicable to animals raised at other temperatures because all developmental events are compressed or expanded proportionally, depending upon the temperature.

However, as pointed out by Bentley *et al.* (1979), a staging system based on percent of total time of embryogenesis cannot be applied in species with a period of developmental arrest. *Homarus* embryos manifest a period of arrested development in natural conditions (Perkins, 1972) and at constant temperature (present study) and these results indicate that factors other than temperature have a strong influence on the rate of embryonic development in lobsters. Therefore, we have used a morphometric index, the size of the screening pigment spot in the lateral eyes (the eye index of Perkins, 1972), as the basis for a percent-staging scheme. The eye index has been used as an indicator of developmental stage in a number of embryonic studies (Schuur *et al.*, 1976; Hepper and Gough, 1978; Cole and Lang, 1980; Sasaki, 1984; Sasaki *et al.*, 1986; Beltz and Kravitz, 1987; Beltz *et al.*, 1990; Helluy and Beltz, 1990; Meier and Reichert, 1990).

There are two obvious limitations of a staging system based upon the eye index. First, the eye pigment does not appear until approximately three weeks after egg extrusion, and at the first possible measurement is about 70 μm. To calibrate the relatively brief period from extrusion to appearance of eye pigment, a time-staging analysis has been used (see Materials and Methods). Thus, a percent-staging system has been established that covers the entire embryonic period, from extrusion until hatching. The second drawback of the proposed method is related to the variation of the eye index at hatching. Perkins (1972) reported that the eye index at hatching is 560 μm. Just prior to hatching in four broods that we have examined, it ranged from 570 μm to 586 μm and, taking into account Perkins' figure of 560 μm, 570 $\pm$ 20 μm was chosen as the eye index at hatching. This figure was used as the end point in establishing the percent-staging scale. The 20 μm variation represents a small proportion (3.5%) of the total percentage scale. The variation of the eye index at hatching could be due to a combination of factors such as genetic variability or perturbations due to environmental conditions. For instance, it has been reported that molting and development of morphological characteristics proceed somewhat independently in decapods (Gore, 1985), and it is conceivable that the physiological changes that regulate hatching and molting could be advanced or delayed with respect to morphogenesis and growth.

Growth curves and the developmental plateau

Lobster egg masses kept at seasonal water temperature show a developmental plateau when the temperature is low during the winter months (Perkins, 1972). More surprising is the fact that our experimental brood, which was

Figure 5. Ventral view of embryos of *Homarus americanus* dissected from the yolk and unfixed at (A) 10, (B) 20, (C) 30, (D) 40, (E) 50, (F) 60, (G) 70, (H) 80, and (I) 90% development. In all photographs, the head is at the top and the abdomen is folded ventrally onto the thorax. The figures in the lower left corners refer to the percentage of development. The line drawing (J) represents a schematic metanauplius. Abbreviations, ab: abdomen, an1: antennula, an2: antenna, ch: chromatophores, he: heart, ig: intestinal granules, le: lateral eye, me: median eye, mxp3: third maxilliped, te: telson. Scale bar in (F), valid from (A) to (I): 500 μm.

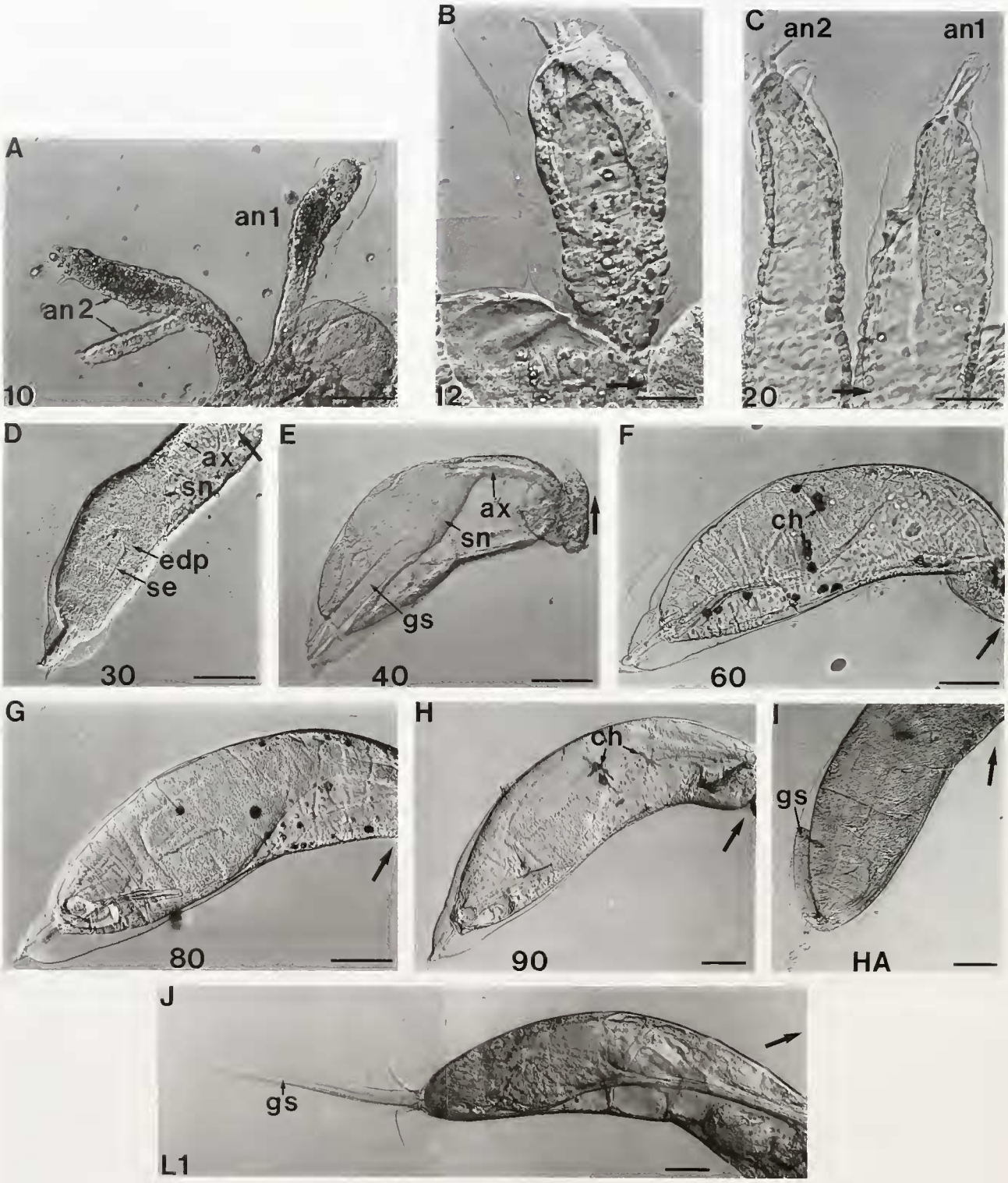

Figure 6. Unfixed antennulae of embryos of *Homarus americanus* at (A) 10, (B) 12, (C) 20, (D) 30, (E) 40, (F) 60, (G) 80, and (H) 90% development, in (I) a hatchling (HA) and in (J) a first larval stage (L1). In all photographs, the figures in the lower left corners refer to the percentage of development. Thick arrows point at the anterior end of the animals. The antenna (an2) is also shown in (A) and (C) in addition to the antennula (an1). By 30% development (E30%), a cluster of presumed sensory neurons (sn) and their axons (ax) are present in the antennula and are particularly visible at E40%. At E30%, the endopod (edp) of the antennula and the seta (se) at its tip are growing: the organogenesis of the first larval stage has already started. The endopod

raised at constant temperature, also showed a developmental plateau. The arrest in development characterized by a lack of growth in either the eye index or the cephalothoracic length, occurred at E82% development (EI 474). At E80%, the eggs are green and the metanauplii are still at stage D_0, whereas by E90% eggs are yellowish and have entered stage D_1. The heterogeneity of the egg population and the changes in pace of development observed in the experimental brood prior to hatching were therefore related to the transition from D_0 to D_1 of the metanaupliar molt cycle.

Developmental plateaus also occur during stage D_0 of the molt cycle in juvenile lobsters (Aiken, 1973). The arrest in development takes place at different times during stage D_0, from the first indication of epidermal retraction to maximal epidermal retraction. Lobsters pause in their development during the cold winter months, and the transition to the irreversible stage D_1 does not proceed until the water warms up in the spring. Aiken (1973) shows that when a lobster has passed beyond pleopod stage 2.5—and therefore entered stage D_1—development then proceeds at a rate regulated by temperature. It is possible that the embryonic metanauplius goes through the same cycle. Indeed, developmental plateaus have been observed at different eye indices during stage D_0 (from 350 μm to 450 μm) in different broods of eggs (Thomann, Beltz, and Helluy, unpub. results). Additionally, Perkins (1972) notes that during the winter months development is arrested in older eggs (extruded early in the summer) and still continues in younger eggs extruded later. It appears, therefore, that in the wild the eggs may spend a variable amount of time in stage D_0 (shorter in younger eggs); in the spring, internal and external cues could trigger the transition from D_0 to D_1. This could explain why extrusion of eggs is a prolonged event in a population of females in the wild, whereas hatching occurs during a more limited period (Herrick, 1895; Perkins, 1972).

Embryonic molt cycles

In the present study, evidence is presented for two molts occurring in *Homarus* prior to the first larval stage and associated with the beginning and the end of the embryonic metanaupliar stage. Other crustaceans are also known to pass through molt cycles within the egg envelopes (Wear, 1974; Goudeau, 1976; Goudeau and Lachaise, 1983). Graf (1972) characterizes the embryonic molt cycle of an amphipod on the basis of changes in the epidermis, setae, and calcium storage; these changes parallel those occurring during juvenile and adult molt cycles. In *Homarus* both Herrick (1895) and Bumpus (1891) mention the existence of embryonic molts. Based on the number of membranes enclosing the embryo, Herrick (1895, p. 183) presumes that at least three embryonic molts have occurred by the time the pigment appears in the lateral eyes and predicts that many more may take place during the long embryonic life. Bumpus (1891) notes that the cuticle lifts from the embryo in the region of the compound eye between stages N and O, and that a true ecdysis follows; this molt is probably the same as that observed in the present study around E12%, which is slightly after stage O of Bumpus. Goudeau *et al.* (1990), using electron microscopy, detect five envelopes originating from the embryo of *Homarus gammarus* secreted beneath the inner and outer egg envelopes and show that the secretion of the embryonic envelopes is associated with high titers of ecdysteroids; however, the embryos are not staged and the timing of secretion is not studied. The fact that the metanaupliar cuticle that begins to lift from the telson at 30% development possesses the imprint of the 6 + 6 setae that were present at the naupliar molt, and the fact that this same metanaupliar cuticle with the imprint of the 6 + 6 setae is discarded at the metanaupliar molt, demonstrate that there is only one instar during that period, not the many that were predicted by Herrick (1895). The progressive setal changes observed in the metanaupliar telson also support this conclusion. The several envelopes seen at the level of the antennulae (Fig. 6B) and antennae at stage E12% may indicate that additional molts occur prior to E12%, during the naupliar phase.

Whereas the setal changes occurring in the telson of the metanauplius seem very similar to those occurring during the molt cycle of larval and juvenile lobsters (Aiken, 1973; Rao *et al.*, 1973; Aiken, 1980; Sasaki, 1984), the cellular and biochemical changes in the epidermis and cuticle must be somewhat different. In the growing metanauplius there is no fixed postecdysial volume as there is in postembryonic animals. Indeed, the cephalothorax of the embryo grows by a factor of about 4 from the early 12% molt to the hatch molt (Fig. 2). Therefore, we presume that there is no mineralization of the metanaupliar cuticle. In that respect, it has also been noted before (see review in Gore, 1985) that the prezoeal cuticle is different from the exuvia of older lobsters.

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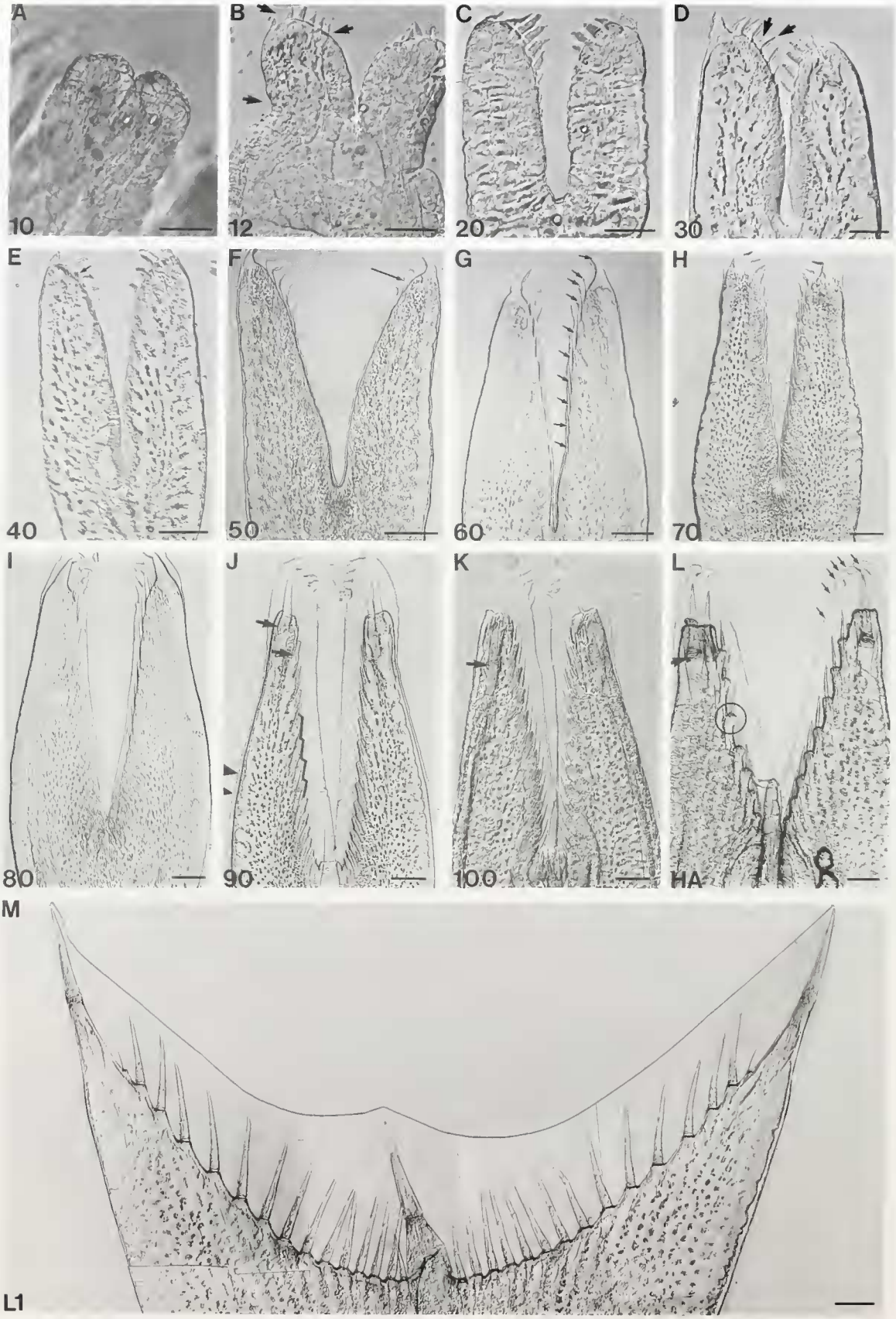
of the antennula is also seen under the cuticle of the metanauplius at E80%, at E90%, and in the hatchling but is out of focus in the photographs of other stages. The giant sensillum (gs) at the tip of the exopod of the antennula is clearly seen forming inverted at E40% development, everted in the hatchling under the cuticle of the metanauplius, and free and erect with three other setae at the tip of the exopod in the antennula of the first larval stage. The red pigment in the chromatophores (ch) is seen concentrated at E60% and E80% and dispersed at E90%. Scale bars, A: 100 μm, B and C: 50 μm, D to J: 100 μm.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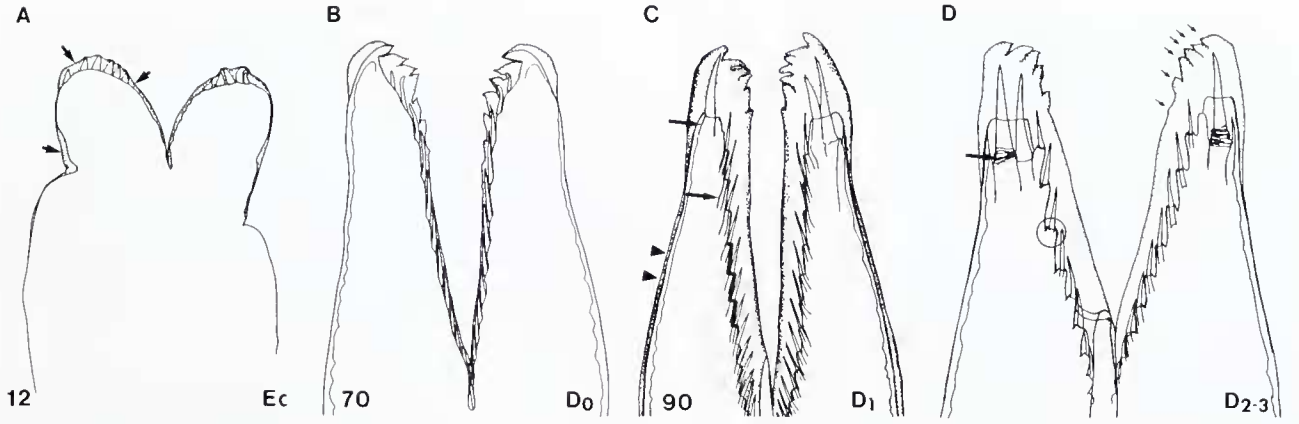

Figure 8. Line drawings of telsons of *Homarus americanus* at (A) 12, (B) 70, (C) 90% development, and (D) in a hatchling showing the naupliar ecdysis (Ec) and representative stages D_0, D_1, D_{2-3}. For photographs of those stages and legend, see Figure 7.

Lobster embryonic development in perspective

In the present study, the metanaupliar molt cycle and the organogenesis of the first larval stage of *Homarus* are examined. This aspect of development is generally ignored in the literature. For example, Bumpus (1891) studies the early embryology of *Homarus* only until stage R (between E30% and E40% development). Herrick (1895, p. 209) implies that the organogenesis of the L1 is extremely brief and takes place just prior to eclosion when he observes that the antennulae "remain single until just before the time of hatching when the inner branch of the flagellum begins to grow." In the present study the endopod (inner branch of the flagellum) of the antennulae is first seen at about 30% development. Therefore, our examination of both the antennulae and the telson indicate that the organogenesis of L1 begins early and continues throughout the embryonic molt cycle.

Overlooking the embryonic metanaupliar molt cycle has led to some confusion as to the status of the prezoea (prelarval form). The existence of a prezoea has been noted in many families of decapods, but its significance has been largely debated (see review in Gore, 1985). We agree with Wear (1974), who observes that "in decapods which hatch at a zoea stage, the prezoeal cuticle is associated with the metanauplius stage relegated to embryonic life, rather than to the preceding nauplius." This is clearly the case in *Homarus:* the ephemeral prelarva (prezoea) *is* the mature metanauplius between the moment it is freed of the two external egg envelopes and the time it molts (Fig. 4D).

Figure 7. Telsons of embryos of *Homarus americanus* (unfixed) at (A) 10, (B) 12, (C) 20, (D) 30, (E) 40, (F) 50, (G) 60, (H) 70, (I) 80, (J) 90, (K) 100% development, in (L) a hatchling (HA), and (M) a first larval stage (L1). Panel M is a montage. In all photographs, distal is at the top. The figures in the lower left corners refer to the percentage of development. The tegumentary and setal changes typical of different stages of the molt cycle as described by Aiken (1973 and 1980), Rao *et al.* (1973), and Sasaki (1984) in larval, juvenile, and adult lobsters are indicated with an asterisk in the following text. At about 12% development (E12%), an embryonic exuvia is lifting from the telson of the nauplius (arrows). The telson of the metanauplius forming under that exuvia is provided with 6 + 6 setae. At E30%, the metanaupliar cuticle begins to separate from the side of the setae* but the tips of these setae are still attached to the cuticle (arrows); this stage is equivalent to the premolt stage D_0 of the molting cycle of older lobsters. During the metanaupliar molt cycle, the setae present on the triangular telson of the first larval stage (first zoea) are forming gradually, proximally and medially in the telson of the metanauplius. By E60% at least 10 + 10 setae (arrows) are visible under the metanaupliar cuticle. At E70%, the full complement of setae of the first larval stage (15 + 15) is formed. Between E80% and E90%, dramatic setal and tegumentary changes occur. At E90% the epidermis is scalloped* (black line); setae and lateral spines are invaginating* (arrows) and the cuticle lifts from the sides of the telson (arrowheads); this stage is equivalent to D_1. Just prior to hatching (E100%) retraction of spines and setae is maximum. Note the crumpled tissue at the base of the lateral spines (arrow). In the hatchling (HA), this tissue forms a dark ring (arrow), the lateral spines are half extended, the epidermis is very distinct* and the bulging of the epidermis around the setae is pronounced* (circle) which is characteristic of stage D_{2-3}. Ecdysis takes place thereafter, and the metanaupliar cuticle bearing the shape of the 6 + 6 metanaupliar setae (arrows) is shed. After ecdysis the triangular telson of the first larval stage (L1) unfolds. Note that two individuals (J and L) had two lateral spines on one side. Scale bars, A to D: 50 μm, E to M: 100 μm.

One interpretation of the coupling of hatching and molting is that hatching is actually a by-product of the molting process. For instance, the extension of the lateral spines of the telson (Fig. 7L) could provoke the breaking of the inner and outer egg envelopes (Fig. 4C, D). Indeed, the lateral setae of the telson are smooth and extended until 80% development (Fig. 7I), begin to invaginate as soon as they become sharp (Figs. 7J, 8C), are entirely invaginated in the blue embryo (E100%) just prior to hatching (Fig. 7K), and are seen half evaginated in the hatchling (Figs. 7L, 8D) whose telson has just pierced the egg envelopes (Fig. 4C). No other part of the mature metanauplius is quite as hard and sharp as the lateral spines of the telson and it is therefore possible that the extension of these spines triggers the rupture of the egg envelopes. By this means, the metanauplius at the end of its molt cycle would precipitate hatching, and the beating of the pleopods of the mother would help the larva to slip out of its swaddling envelopes.

Molt cycles in postembryonic crustaceans are under hormonal control, and it is likely that embryonic molt cycles are regulated in a similar way. The circulating molting hormone is an ecdysteroid whose titers increase in D_0 and peak in D_2–D_3 in *Homarus* (Snyder and Chang, 1991) and in D_1 in *Penaeus* (Chan *et al.*, 1988). Because steroids influence neuronal development and survival in other systems (Weeks and Truman, 1986), the awareness of embryonic molts and the prediction of the timing of potential changes in steroid levels could be critical for future developmental neurobiological studies.

The nauplius is a form common to all crustaceans, and in some taxa (*e.g.*, Cirripedia, Anostraca) eggs hatch as naupliі. In other species, the naupliar stage is followed within the egg envelopes by the organogenesis of a more complex body form characterized by the morphogenesis and growth of the postmandibular region (Anderson, 1979, 1982; Weygoldt, 1979; Williamson, 1982; Gore, 1985; Schram, 1986; Shiino, 1988). It has been shown in several taxa [amphipods: Graf (1972); isopods: Goudeau (1976); decapods: Wear (1974), and the present study] that envelopes equated to embryonic exuvia are found during embryonic life as the postmandibular region differentiates. In amphipods (Graf, 1972) and decapods (present study), embryonic molt cycles are demonstrated with the progressive setal and tegumentary changes occurring in the telson. The existence of embryonic molt cycles in different taxa suggests that the relegation of larval stages to life in the egg, or, rather, the delay of hatching with regard to molting, is a widespread and distinctive evolutionary strategy in crustaceans. Besides leading to evolutionary considerations, the characterization of the metanaupliar molt cycle and the percent-staging scheme for lobster eggs should lend added insight in future investigations of neural, physiological, and ecological aspects of *Homarus* embryonic life.

Acknowledgments

We wish to thank Maureen Ruchhoeft for her kind and skillful help, Joe Gagliardi and Kay Leland for printing photographic plates, Michael Syslo and Kevin Johnson from the Massachusetts State Lobster Hatchery who provided the egg-bearing female lobsters, as well as Colleen Boggs and Tom Coffee who maintained them at the New England Aquarium. (Supported by NSF-BNS-8718938, NIH-NS 25915, and NSF- Presidential Young Investigator Award BNS- 8958169 to B.S.B.)

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